

Ether Injection-Based Formulation and *In-Vitro* Characterization of Atorvastatin-Encapsulated Niosomes: A Smart Nanocarrier for Enhanced Drug Delivery

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DOI: <https://doi.org/10.46382/mjbas.2025.9401>



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Article Received: 03 September 2025

Article Accepted: 07 November 2025

Article Published: 09 November 2025

ABSTRACT

Atorvastatin calcium, a BCS Class II medication for hyperlipidemia, has only 14% oral bioavailability due to its poor permeability and significant presystemic metabolism by the liver as well as the gut wall. To enhance its oral bioavailability, this study aimed to prepare six distinct formulations of atorvastatin loaded niosomes utilizing the Tween 20 by ether injection technique and to evaluate their *in vitro* profile indulging the drug content and the pattern of drug release. These results are compared, a standard calibration curve with R² value of 0.9946, with marketed three branded medications. The surprising formulation is found to be F6, which had a medication, surfactant, and cholesterol ratio of 1:3.5:1 and showed a maximum potency of 116.95%, surpassing all marketed samples, S1-S3, where S2 was highest at 103.3%, and an 80.12% drug release within 90 minutes. The enhanced drug release and potency imply that the F6 formulation presents a strong possibility for enhanced therapeutic efficacy, hence establishing it as a feasible contender for more research and clinical implementation.

Keywords: Atorvastatin; Bioavailability; Dissolution; Drug Content; Ether Injection; Formulation; *In-Vitro*; Niosomes; Nanocarrier; Tween.

1. Introduction

Atorvastatin is used to reduce blood cholesterol levels [1]. It is classified as a Class II biopharmaceutical medication due to low solubility (< 1 mg/ml), limiting its release from the formulation [2,3]. It is largely absorbed by the liver, resulting in inadequate systemic bio-availability (14%) due to first-pass metabolism by the liver [4].

Historically, the preferred route was orally administering medications [5]. Most of the discovered and commonly used drugs taken orally may have bio-availability issues for a variety of reasons, including poor solubility, low permeability [6]. There are several basic approaches to alter the solubility and permeability of weakly water-soluble medicament such as atorvastatin [4]. Niosomes qualify unique reward over traditional dosage forms, as they can do work as reservoirs to encapsulate and release drugs [7] and demonstrate crucial amenities as drug carriers, particularly due to their chemical stability [8-9].

1.1. Study Objectives

- 1) Prepare six distinct formulations of atorvastatin loaded niosomes utilizing the Tween 20 by the ether injection technique.
- 2) Evaluate the drug content and the drug release pattern of atorvastatin loaded niosomal formulations.
- 3) Evaluate the drug content and the drug release pattern of marketed atorvastatin formulations.
- 4) Comparative study of dissolution profile and drug content among six formulations and three marketed samples.

2. Materials and method

2.1. Materials

Atorvastatin calcium (Beacon Pharmaceuticals, Bangladesh), Cholesterol, Tween20, Chloroform, Phosphoric acid and hydrochloric acid, were of analytical grade. UV spectrophotometer, Dissolution Tester, Electronic balance, facilitated by the analytical laboratory of Bangladesh University, was used during the whole process.

2.2. Ether Injection method

Atorvastatin was dissolved in chloroform to make the solution-I and another solution-II was obtained by mixing Tween 20 and cholesterol together. Via microsyringe solution-I added to solution-II at 1 mL/min and continually stirred on water bath of 55-60 °C resulting the niosomal atorvastatin formulation [10-11].

2.3. Calibration curve preparation

The volume was adjusted by 0.1N HCl of 100 mL volumetric flask containing 10 mg of Atorvastatin calcium. Then 10 mL filtered solution was transferred and diluted adding distilled water to be the final volume of 100 mL resulting the 10 µg/mL concentration of the solution. The stock solution was then successfully diluted in five distinct test tubes to make different concentration of the stock solution. Then the absorbance of these distinct solutions was being measured by UV-Vis spectroscopy at 246 nm. These absorbance measurements and concentrations value were graphed to develop a standardization curve of $y = 0.0165x + 0.1421$ with $R^2 = 0.9946$ [12-13].

2.4. Dissolution studies

The drug release study evaluated the percentage of drug released into the dissolving media terminated in a given period providing an estimated amount of drug available for absorption after oral administration. Holding appropriate dissolving properties is critical for a good tablet. The in vitro release of marketed atorvastatin and niosomal atorvastatin formulation was investigated through the egg membrane in the USP dissolution apparatus II at $37 \pm 0.2^\circ\text{C}$ using 0.1N HCl buffer. After 15, 30, 45, 60, 75, and 90 minutes, a 10 mL sample was taken each slot from the medium and absorbance of these filtrate were measured at 246 nm in a UV-Vis spectrophotometer [11].

2.5. Drug content

Three brands of Atorvastatin tablets (10 mg) was collected from a model pharmacy in Dhaka. Ten tablets of from each one brand were weighed and smashed. Equivalent amount of 10 mg atorvastatin calcium, crushed powder, was added in a volumetric flask. Then it filled up with 0.1N HCl up to 100 mL. The resultant solution was filtered. Then 10 mL of filtrate was being thinned with 90 mL distilled water. Finally each solutions absorbance was calculated by UV-Vis spectrometry at 246 nm [14].

3. Result and Discussion

Six niosomal formulations were developed to determine an optimum formula using in-vitro methods and the proportions of drug, surfactant and cholesterol for the preparations are shown in Table 1. In this investigation, we explored how varied amounts of Tween 20 surfactants affected the percentage of medication released in six formulations of atorvastatin-loaded niosomes.

Table 1. Constituents of the atorvastatin loaded niosomal formulation

Formulation Code	Atorvastatin (mg)	Tween 20 (mg)	Cholesterol (mg)	Ratio	Chloroform (ml)	Total volume (ml)
F1	100	100	100	1:1:1	100	300
F2	100	150	100	1:1.5:1	100	350
F3	100	200	100	1:2:1	100	400
F4	100	250	100	1:2.5:1	100	450
F5	100	300	100	1:3:1	100	500
F6	100	350	100	1:3.5:1	100	550

To evaluate the drug release pattern and potency, it was crucial to develop a standard calibration curve of atorvastatin (Figure 1) and the data were inscribed in it. We created a standard calibration curve to assist in the calculation of dissolution data for both niosomal preparations and commercial pharmaceuticals.

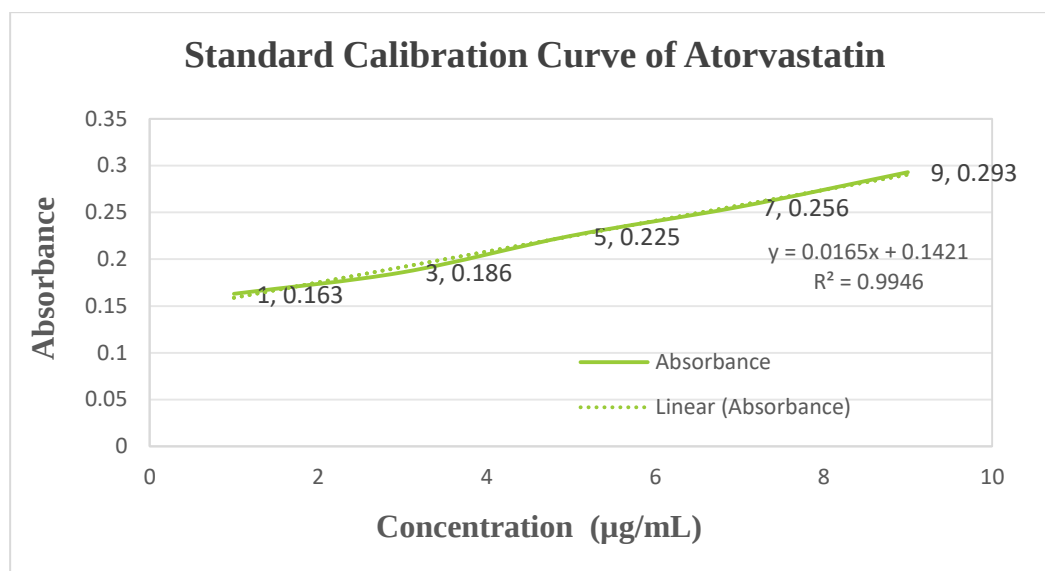


Figure 1. Standard Calibration Curve of Atorvastatin

Table 2 illustrates the proportion of drug release data, allowing for a comparative analysis of niosomal formulations (F1-F6) and marketed samples (S1-S3). The Figure 2 developed from this data shows drug release percentages for the six formulations, which aids in determining which one is best. The F1 formulation exhibited a steady increase in drug release, with a modest increase after 90 minutes. In contrast, the F2 formulations showed a continuous rise in drug dissolution, peaking at 75 and 90 minutes. Formulations F3 through F6 showed moderate increases in dissolution values, with F3, F4, and F6 achieving the highest levels. The F6 formulation, for example, achieved 80.12% drug release after 90 minutes. We also measured the disintegration times of three sample medications and compared them ranging from 15 to 90 minutes. All samples met USP standards (100-150%) for active components. Sample 2 had a maximum release time of 60 minutes, reaching 140.1%. Our F6 formulation displayed improved solubility within 90 minutes, indicating the possibility of higher atorvastatin bioavailability. We subsequently compared our formulations to three commercially available medications.

Table 2. Comparative study of dissolution profile among six formulations and three marketed samples

Formulation	Time for percentage of drug release					
	15	30	45	60	75	90
F1	2.63	4.85	7.58	9.22	12.49	13.58
F2	7.58	11.94	19.03	21.76	31.03	38.12
F3	12.48	20.12	30.48	39.21	45.75	50.12
F4	15.75	23.94	27.12	43.02	50.12	52.84
F5	11.94	13.58	22.30	27.21	33.75	35.93
F6	14.67	25.02	40.30	54.48	59.94	80.12
S1	102.5	101.4	111.8	134.7	134.9	135
S2	112.3	130.3	137.9	140.1	140.3	140.5
S3	110.7	116.7	123.8	129.8	130	130.2

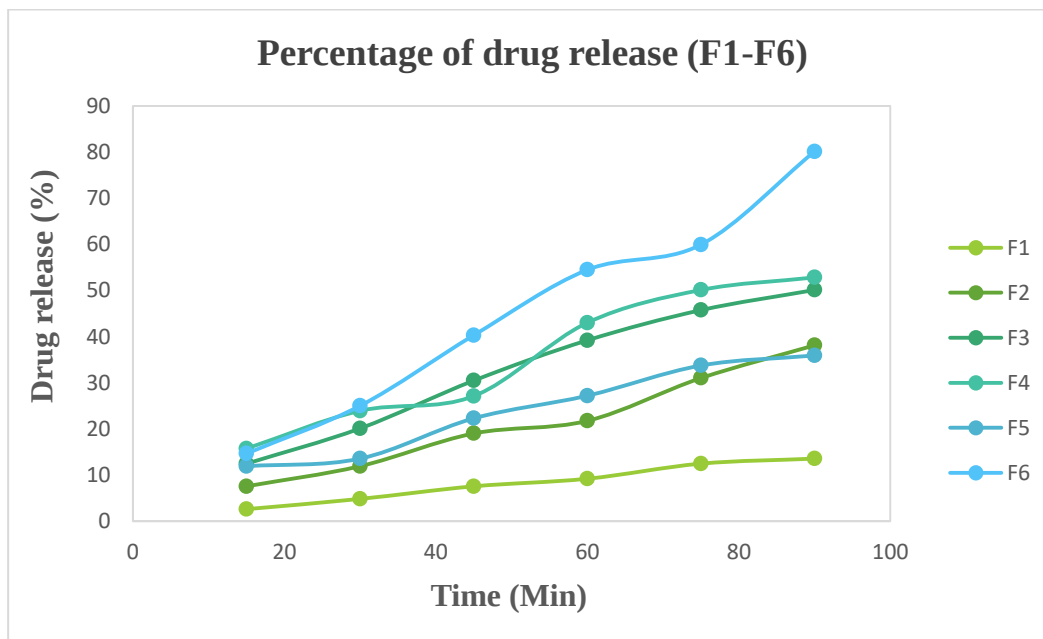


Figure 2. Percentage of drug release (F1-F6)

In addition, we evaluated the potency of our niosomal formulations with three different commercial atorvastatin tablets, with the goal of increasing atorvastatin solubility and dissolution rates. The table 3 summarizes the potencies of the marketed medications, with S2 having the highest potency (103.3%). We compared the potency of our formulations to three commercial medicines. F3, F4, and F6 formulations met USP standards ($100 \pm 15\%$) with potencies of 102.42%, 110.38%, and 116.95%, respectively. This comparison in Figure 3 showed that F3, F4, and F6 formulation potencies exceeded those of the marketed drugs. However, according to USP standards, atorvastatin must dissolve at least 80% within 30 minutes. While the commercial medicine S1-S3 met this condition, the majority of niosomal formulations, except F2, did not meet it. Despite its quick absorption and strong plasma protein binding, atorvastatin calcium has low bioavailability due to insufficient dissolution and substantial first-pass metabolism. Our formulations had variable drug release rates over time, with some

demonstrating moderate release and linearity. The fundamental purpose of the niosomal drug delivery system is to release pharmacological substances in a regulated and targeted manner.

Table 3. Data for potency of formulations and marketed samples

Formulation	Absorbance	Potency (%)
F1	0.198	68.51
F2	0.287	99.30
F3	0.296	102.42
F4	0.319	110.38
F5	0.201	69.55
F6	0.338	116.95
S1	0.297	102.6
S2	0.299	103.3
S3	0.281	97.9

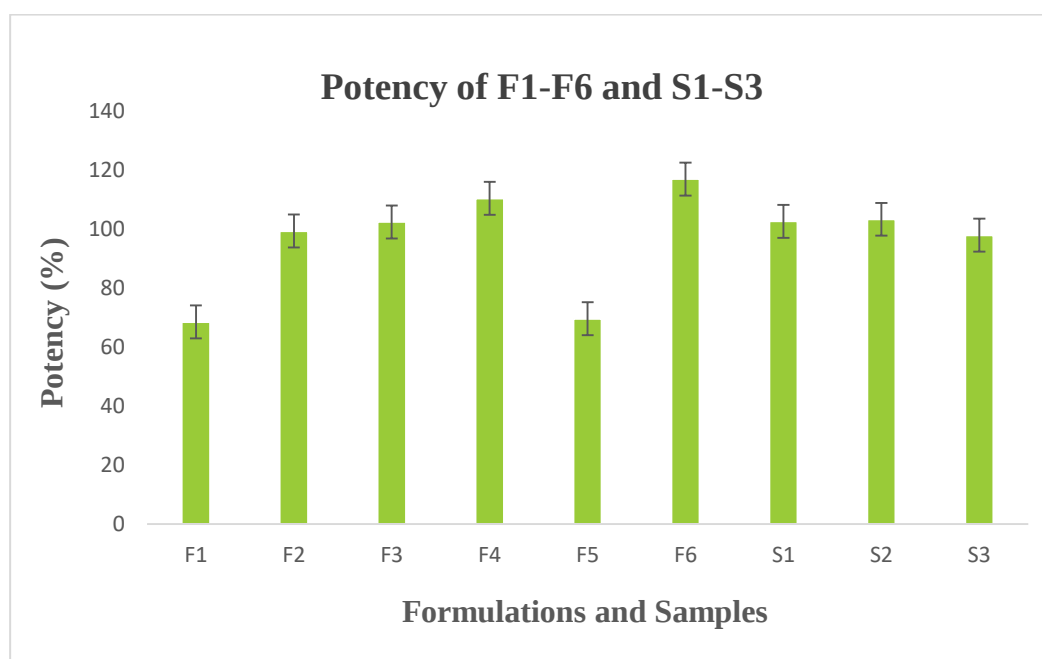


Figure 3. Potency of F1-F6 and S1-S3

4. Conclusion

The niosomal drug delivery system is arguably the best example of major advancements in drug delivery and nanotechnology. Niosomes are an ideal drug delivery technology because of their durability, ability to permeate biological membranes, and high oral bioavailability. Formulations were developed to gain acceptable oral bioavailability of the medication in vivo. Considering the data, it is affirmable that niosomes can be employed to improve atorvastatin oral bioavailability. To provide a more comprehensive assessment of product quality of Atorvastatin loaded niosomes, the following future studies should be investigated:

- 1) Continued study of the ether injection technique to find beneficial formulations.

- 2) Additional in vitro research is required to develop novel formulations.
- 3) In vivo testing is necessary to determine the drug's bioavailability before introducing these formulations.
- 4) Developing controlled and targeted releases for future optimization.
- 5) Exploring the niosomal drug delivery system.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis, and manuscript writing equally.

Availability of data and materials

Supplementary information is available from the authors upon reasonable request.

Institutional Review Board Statement

Not applicable for this study.

Informed Consent

Not applicable for this study.

Acknowledgement

The presenting authors are grateful to the Department of Pharmacy, Bangladesh University, Dhaka for providing the necessary facilities.

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